

AMENDMENTS TO THE SPECIFICATION

IN THE SPECIFICATION:

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Please amend the paragraph beginning at line 4, through page 12, line 24 as follows:

That is, the present invention relates to:

[1] a paste comprising bacteria-CWS which consists of a bacteria-CWS and an oil wherein the paste has a viscosity of 0.7 poise or less (25 °C);

[2] the paste comprising bacteria-CWS according to [1] wherein the paste has a viscosity between 0.2 and 0.7 poise (25 °C);

[3] the paste comprising bacteria-CWS according to [1] wherein the paste has a viscosity between 0.28 and 0.55 poise (25 °C);

[4] the paste comprising bacteria-CWS according to any one of [1] to [3], wherein the particle diameter of the bacteria-CWS is between 0.15 µm and 6 µm;

[5] the ~~paste~~ comprising bacteria-CWS according to [4], wherein D10 % of 0.38 µm or more, and D90% of 0.70 µm or less for the particle diameter of the bacteria-CWS;

[6] the paste comprising bacteria-CWS according to any one of [1] to [5], wherein the bacteria-CWS is BCG-CWS;

[7] the paste comprising bacteria-CWS according to any one of [1] to [6], wherein an oil is selected from the group consisting of squalane, squalene, synthelane 4, peanut oil, camellia oil, soybean oil, liquid paraffin, and ethyl oleate or a mixture of one or more oils thereof;

[8] the paste comprising bacteria-CWS according to [7] wherein the oil is a mixture of two types of oils selected from the group consisting of squalane, squalene, soybean oil, liquid paraffin, and ethyl oleate;

[9] the paste comprising bacteria-CWS according to [8] wherein the oil is a mixture of an oil selected from the group consisting of soybean oil, liquid paraffin and ethyl oleate in admixture with squalane;

[10] the paste comprising bacteria-CWS according to [9], wherein the oil is a 1:1 mixture of ethyl oleate and squalane;

[11] the paste comprising bacteria-CWS according to [7] wherein the oil is squalane;

[12] the paste comprising bacteria-CWS according to any one of [1] to [5], wherein the bacteria-CWS is BCG-CWS and wherein the paste comprises 6.6 g to 35.2 g of squalane per about 0.67 g of BCG-CWS;

[13] a process for preparing a paste comprising bacteria-CWS, which comprises the following steps:

(1) a step of mixing the bacteria-CWS and an oil in an organic solvent as used as a dispersion-aiding solvent; and

(2) a step of removing the organic solvent in (1) by distillation;

[14] the process for preparation according to [13] wherein the organic solvent is an ether solvent;

[15] the process for preparation according to [13] wherein the organic solvent is a hydrocarbon solvent or a halogenated hydrocarbon solvent;

[16] the process for preparation according to [15], wherein the organic solvent is a halogenated hydrocarbon solvent selected from the group consisting of 1,2-dichloroethane, chloroform, and dichloroethane;

[17] the process for preparation according to [15], wherein the organic solvent is a hydrocarbon solvent selected from the group consisting of heptane and hexane;

[18] the process for preparation according to any one of [15] to [17], wherein the organic solvent comprises 5 to 20 % (v/v) of an alcohol solvent;

[19] a paste comprising bacteria-CWS obtainable by the process for preparation according to any one of [13] to [18];

[20] the paste according to [19] wherein the bacteria is BCG bacteria;

[21] the paste according to [19] or [20], wherein the oil is squalane or liquid paraffin;

[22] an oil-in-water emulsion which comprises the paste comprising bacteria-CWS according to any one of [1] to [12], and [19] to [21], a surfactant, a stabilizer, and water;

[23] the oil-in-water emulsion according to [22], which comprises 0.66 g to 3.35 g of the bacteria-CWS, and 0.4 wt% to 8 wt% of the oil per 2L of water;

[24] the oil-in-water emulsion according to [22] or [23], which contains 1 to 10 % mannitol as a stabilizer;

[25] the oil-in-water emulsion according to any one of [22] to [24], which comprises 0.01 % to 3% polyethylenexysorbitan fatty acid ester as a surfactant;

[26] the oil-in-water emulsion according to [25], wherein the polyethylenexysorbitan fatty acid ester is Tween 80;

[27] the oil-in-water emulsion according to any one of [22] to [26], having the following properties:

- (1) the particle diameter of an oil droplet of the emulsion is 0.2 to 30 μm ;
- (2) the bacteria-CWS is encapsulated in the oil droplet, and is negative for reaction with lectin;

[28] the oil-in-water emulsion according to [27] wherein D10% is 0.5 μm or more, and D90% is 20 μm or less as for the diameter of the oil droplet of the emulsion;

[29] a process for preparation of the oil-in-water emulsion according to any one of [22] to [28], which comprises the following steps:

(1) a step of emulsifying a mixture comprising the paste comprising bacteria-CWS according to any one of [1] to [12] and [19] to [21], and an aqueous solution containing a surfactant at a temperature higher than the turbidity point; and

(2) a step of adding an aqueous solution containing a stabilizer for dilution;

[30] the process for preparation according to [29], wherein step (2) is conducted at a temperature lower than the turbidity point;

[31] the process for preparation according to [29] or [30] wherein the emulsification step in step (1) comprises the following steps:

(3) a step of emulsifying a mixture comprising the paste comprising bacteria-CWS according to any one of [1] to [12] and [19] to [21], and an aqueous solution containing 0.02 % to 0.8 % of a surfactant (rough emulsification step); and

(4) a step of adding an aqueous solution containing a surfactant to the mixture of (3) to adjust the concentration of the surfactant, and vigorously stirring the mixture (complete emulsification);

[32] the process for preparation according to [31], wherein the surfactant is polyethylene oxysorbitan fatty acid ester;

[33] the process for preparation of the oil-in-water emulsion according to [32], wherein the polyoxyethylene oxysorbitan fatty acid ester is Tween 80;

[34] the process for preparation of the oil-in-water emulsion according to any one of [29] to [33], wherein the stabilizer is mannitol;

[35] a lyophilized formulation obtainable by lyophilizing the emulsion according to any one of [22] to [28];

[36] an assembly of bacterial cell wall skeleton component particles (an assembly of bacteria-CWS particles) wherein the particle diameter is 0.15 to 6 μm in the particle size distribution;

[37] an assembly of bacteria-CWS particles wherein the particle diameter is 0.2 to 2 μm in the particle size distribution;

[38] the assembly of bacteria-CWS particles according to [36] to [37], wherein the particle size distribution shows a single peak, as well as D10 % of 0.2 μm or more and D90 % of 0.7 μm or less;

[39] the assembly of bacteria-CWS particles according to the any one of [36] to [38], wherein the particle size distribution shows a single peak, as well as D10%: 0.23 ± 0.05 μm and D90%: 0.60 ± 0.05 μm ;

[40] a process for preparation of the assembly of bacteria-CWS particles according to any one of [36] to [39], which comprises dispersing the bacteria-CWS in a solvent containing an aliphatic hydrocarbon solvent;

[41] the process according to [40], wherein the solvent is a mixture of an aliphatic hydrocarbon solvent and an alcohol solvent;

[42] the process for preparation according to [41], wherein the solvent is a heptane containing 5 to 20 % ethanol; and

[42-2] a bacteria-CWS obtainable by the process according to any one of [40] to [42].

That is, the present invention relates to:

[1] a paste comprising bacteria-CWS which consists of a bacteria-CWS and an oil wherein the paste has a viscosity of 0.7 poise or less (25 °C);

[2] the paste comprising bacteria-CWS according to [1] wherein the paste has a viscosity between 0.2 and 0.7 poise (25 °C);

[3] the paste comprising bacteria-CWS according to [1] wherein the paste has a viscosity between 0.28 and 0.55 poise (25 °C);

[4] the paste comprising bacteria-CWS according to any one of [1] to [3], wherein the particle diameter of the bacteria-CWS is between 0.15 μm and 6 μm ;

[5] the paste comprising bacteria-CWS according to [4], wherein D10 % of 0.38 μm or more, and D90% of 0.70 μm or less for the particle diameter of the bacteria-CWS;

[6] the paste comprising bacteria-CWS according to any one of [1] to [5], wherein the bacteria-CWS is BCG-CWS;

[7] the paste comprising bacteria-CWS according to any one of [1] to [6], wherein an oil is selected from the group consisting of squalane, squalene, synthelane 4, peanut oil, camellia oil, soybean oil, liquid paraffin, and ethyl oleate or a mixture of one or more oils thereof;

[8] the paste comprising bacteria-CWS according to [7] wherein the oil is a mixture of two types of oils selected from the group consisting of squalane, squalene, soybean oil, liquid paraffin, and ethyl oleate;

[9] the paste comprising bacteria-CWS according to [8] wherein the oil is a mixture of an oil selected from the group consisting of soybean oil, liquid paraffin and ethyl oleate in admixture with squalane;

[10] the paste comprising bacteria-CWS according to [9], wherein the oil is a 1:1 mixture of ethyl oleate and squalane;

[11] the paste comprising bacteria-CWS according to [7] wherein the oil is squalane;

[12] the paste comprising bacteria-CWS according to any one of [1] to [5], wherein the bacteria-CWS is BCG-CWS and wherein the paste comprises 6.6 g to 35.2 g of squalane per about 0.67 g of BCG-CWS;

[13] a process for preparing a paste comprising bacteria-CWS, which comprises the following steps:

(1) a step of mixing the bacteria-CWS and an oil in an organic solvent as used as a dispersion-aiding solvent; and

(2) a step of removing the organic solvent in (1) by distillation;

[14] the process for preparation according to [13] wherein the organic solvent is an ether solvent;

[15] the process for preparation according to [13] wherein the organic solvent is a hydrocarbon solvent or a halogenated hydrocarbon solvent;

[16] the process for preparation according to [15], wherein the organic solvent is a halogenated hydrocarbon solvent selected from the group consisting of 1,2-dichloroethane, chloroform, and dichloroethane;

[17] the process for preparation according to [15], wherein the organic solvent is a hydrocarbon solvent selected from the group consisting of heptane and hexane;

[18] the process for preparation according to any one of [15] to [17], wherein the organic solvent comprises 5 to 20 % (v/v) of an alcohol solvent;

[19] a paste comprising bacteria-CWS obtainable by the process for preparation according to any one of [13] to [18];

[20] the paste according to [19] wherein the bacteria is BCG bacteria;

[21] the paste according to [19] or [20], wherein the oil is squalane or liquid paraffin;

[22] an oil-in-water emulsion which comprises the paste comprising bacteria-CWS according to any one of [1] to [12], and [19] to [21], a surfactant, a stabilizer, and water;

[23] the oil-in-water emulsion according to [22], which comprises 0.66 g to 3.35 g of the bacteria-CWS, and 0.4 wt% to 8 wt% of the oil per 2L of water;

[24] the oil-in-water emulsion according to [22] or [23], which contains 1 to 10 % mannitol as a stabilizer;

[25] the oil-in-water emulsion according to any one of [22] to [24], which comprises 0.01 % to 3% polyethylenoxysorbitan fatty acid ester as a surfactant;

[26] the oil-in-water emulsion according to [25], wherein the polyethylenexorsorbitan fatty acid ester is Tween 80;

[27] the oil-in-water emulsion according to any one of [22] to [26], having the following properties:

- (1) the particle diameter of an oil droplet of the emulsion is 0.2 to 30 μm ;
- (2) the bacteria-CWS is encapsulated in the oil droplet, and is negative for reaction with lectin;

[28] the oil-in-water emulsion according to [27] wherein D10% is 0.5 μm or more, and D90% is 20 μm or less as for the diameter of the oil droplet of the emulsion;

[29] a process for preparation of the oil-in-water emulsion according to any one of [22] to [28], which comprises the following steps:

- (1) a step of emulsifying a mixture comprising the paste comprising bacteria-CWS according to any one of [1] to [12] and [19] to [21], and an aqueous solution containing a surfactant at a temperature higher than the turbidity point; and

- (2) a step of adding an aqueous solution containing a stabilizer for dilution;

[30] the process for preparation according to [29], wherein step (2) is conducted at a temperature lower than the turbidity point;

[31] the process for preparation according to [29] or [30] wherein the emulsification step in step (1) comprises the following steps:

- (3) a step of emulsifying a mixture comprising the paste comprising bacteria-CWS according to any one of [1] to [12] and [19] to [21], and an aqueous solution containing 0.02 % to 0.8 % of a surfactant (rough emulsification step); and

(4) a step of adding an aqueous solution containing a surfactant to the mixture of (3) to adjust the concentration of the surfactant, and vigorously stirring the mixture (complete emulsification);

[32] the process for preparation according to [31], wherein the surfactant is polyethylene oxysorbitan fatty acid ester;

[33] the process for preparation of the oil-in-water emulsion according to [32], wherein the polyoxyethylene oxysorbitan fatty acid ester is Tween 80;

[34] the process for preparation of the oil-in-water emulsion according to any one of [29] to [33], wherein the stabilizer is mannitol;

[35] a lyophilized formulation obtainable by lyophilizing the emulsion according to any one of [22] to [28];

[36] an assembly of bacterial cell wall skeleton component particles (an assembly of bacteria-CWS particles) wherein the particle diameter is 0.15 to 6 μm in the particle size distribution;

[37] an assembly of bacteria-CWS particles wherein the particle diameter is 0.2 to 2 μm in the particle size distribution;

[38] the assembly of bacteria-CWS particles according to [36] to [37], wherein the particle size distribution shows a single peak, as well as D10 % of 0.2 or more and D90 % of 0.7 or less;

[39] the assembly of bacteria-CWS particles according to the any one of [36] to [38], wherein the particle size distribution shows a single peak, as well as D10%: 0.23 ± 0.05 and D90%: 0.60 ± 0.05 ;

[40] a process for preparation of the assembly of bacteria-CWS particles according to any one of [36] to [39], which comprises dispersing the bacteria-CWS in a solvent containing an aliphatic hydrocarbon solvent;

[41] the process according to [40], wherein the solvent is a mixture of an aliphatic hydrocarbon solvent and an alcohol solvent;

[42] the process for preparation according to [41], wherein the solvent is a heptane containing 5 to 20 % ethanol; and

[42-2] a bacteria-CWS obtainable by the process according to any one of [40] to [42].

Further, in this embodiment, the present invention relates to:

[42-3] the paste comprising bacteria-CWS according to the present invention which comprises an assembly of bacteria-CWS particles, wherein the particle diameter is from 0.1 μm to 20 μm , preferably from 0.15 μm to 6 μm , and more preferably 0.2 μm to 2 μm ;

[42-4] the paste comprising bacteria-CWS according to [42-3], wherein the assembly of bacteria-CWS particles exhibit a particle size distribution showing a single peak as well as D10%: $0.23 \pm 0.05 \mu\text{m}$ and D90%: $0.60 \pm 0.05 \mu\text{m}$;

[42-5] an oil-in-water emulsion which comprises the paste comprising bacteria-CWS according to [42-3] or [42-4], a surfactant, a stabilizer, and water;

[42-6] a lyophilized formulation obtainable by lyophilizing the emulsion according to [42-5]; and

[42-7] a pharmaceutical composition which consists of the emulsion of the present invention.

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Please amend the paragraph beginning at line 15, through line 16 as follows:

Figure 2 shows the particle size distribution of BCG-CWS in the paste using 90 % heptane/10 % ethanol as a dispersion-aiding solvent.

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Please replace the paragraph beginning at line 22, through Page 26, line 1 as follows:

Preferred pastes comprising bacteria-CWS are those comprising an assembly of bacteria-CWS particles wherein the particle diameter is 0.1 μm to 20 μm , preferably 0.15 μm to 6 μm , more preferably 0.2 μm to 2 μm in the particle size distribution, and those wherein the particle size distribution shows a single peak, and D10 % of 0.23 ± 0.05 μm and D90 % of 0.60 ± 0.05 μm .

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Please amend Table 2 as follows:

Table 2

	BCG-CWS	Oil (8.4 g)	Viscosity (poise)
1	670 mg	Soybean oil	6.95
2	670 mg	Squalane	0.55
3	670 mg	Synthelane 4	0.49
4	<u>670 mg</u>	Drakeol	0.28
5	670 mg	Ethyl oleate	0.11

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Please amend the paragraph beginning at line 3, through line 5 as follows:

| *~~SMP-105~~BCG-CWS concentration of the suspension is expressed as percentage based
on the desired concentration. The amount of adhesion is reported in parentheses.

Please amend the paragraph beginning at line 7, through line 9 as follows:

| *~~SMP-105~~BCG-CWS concentration of the suspension is expressed as percentage based
on the desired concentration. The amount of adhesion is reported in parentheses.